

COMPARATIVE STUDIES OF THE OXYGEN CONSUMPTION OF
THREE SPECIES OF NEOTENIC SALAMANDERS AS INFLUENCED
BY TEMPERATURE, BODY SIZE, AND OXYGEN TENSION¹

W. E. NORRIS, JR.,² PAUL A. GRANDY³ AND W. K. DAVIS

*Biology Department, Southwest Texas State College, San Marcos, Texas, and
Marine Biological Laboratory, Woods Hole, Massachusetts*

As a result of the mechanism of natural selection, populations of organisms have arisen that exhibit different degrees of physiological adaptation. Numerous facets of this subject have been discussed extensively in review papers by Prosser (1955) and by Bullock (1955).

Vernberg (1959a, 1959b) has recently investigated physiological variations in latitudinally isolated populations of fiddler crabs. As a result of these studies he has suggested that the metabolic response of the organisms has real significance as to their distribution, and points out that in the course of evolution the various populations that he studied appeared to be metabolically adjusted to the temperature fluctuation of their habitat. This problem of variation in several different marine animals, collected at different latitudes, has also been studied by Fox and Wingfield (1937), and many others.

Bullock (1955) makes clear that it is not necessary for wide geographical gaps to exist between organisms in order for them to show significant physiological variations, which in turn may affect their distribution. Illustrative of this point is the recent study of Wiens and Armitage (1961) conducted on two species of crayfish (one of which is an inhabitant of permanent ponds and running streams, and the other of temporary roadside ditches) that exist in the same area. The two species are rarely found in association with one another.

At the present time there is keen interest in the value of physiological studies in providing a greater depth of understanding of some of the problems of evolution, ecological distribution, and speciation. Prosser (1955) has called attention to the fact that (p. 229) "comparison of physiological adaptations should contribute much to an understanding of interspecific relations, intraspecific variation, and the bases for ecological ranges."

The literature on salamander respiration is not extensive. Vernberg (1952; see this paper also for earlier references) measured oxygen consumption of two species of salamanders at different seasons of the year at two different temperatures. He found seasonal differences to occur, although a correlation with habitat temperature was not clear. Species differences were noted. Much earlier, Evans (1939) had examined various factors influencing the oxygen consumption of

¹ Supported by state-appropriated funds for organized research.

² This report was prepared at the Marine Biological Laboratory, Woods Hole, Mass., during the summer of 1963 while the senior author was a National Science Foundation, Science Faculty Fellow.

³ Present address: University of Texas, Medical Branch, Galveston, Texas.

several species of plethodontid salamanders. He was able to correlate activity of a species with habitat and oxygen consumption, and in some cases he noted that oxygen consumption was dependent on oxygen tension. Several investigations of the metabolic rate of salamanders during embryogenesis have been reported (Løvtrup and Werdinius, 1957; for review of this literature see Boell, 1955).

The Balcones escarpment region of the Edwards plateau of south central Texas is occupied by several forms of neotenic salamanders, five of which belong to the genus *Eurycea* (Baker, 1961). Three species of this genus are restricted in distribution to certain specific areas along the escarpment. The possibility exists that the different environmental conditions required by these three forms may be related to their individual metabolic requirements. This report deals with a study of oxygen consumption of these salamanders as influenced by temperature, body size, and oxygen tension; and was originally undertaken with the idea that the data obtained might correlate with the restricted distribution of the organisms.

METHODS AND MATERIALS

The three species of salamanders used in this investigation were collected from their native habitat (Table III) and were transported to the laboratory in glass aquaria filled with water and aquatic vegetation collected with the animals. The specimens were maintained in an air conditioned laboratory ($23 \pm 2^\circ \text{C.}$) until respiratory measurements were made. The salamanders were not fed (other than the food material present in the water and vegetation collected with them), and were never kept in the laboratory for longer than a week. Respiratory measurements were frequently made on the same animal more than once.

All oxygen consumption measurements were made with the Warburg constant volume respirometer. The direct method was employed, as described by Umbreit *et al.* (1957). In addition to KOH as a CO_2 absorbent in the center well, each reaction vessel contained three milliliters of water taken directly from the aquaria in which the salamanders were maintained. Control experiments established the fact that neither pH nor nitrogen content of the water changed significantly during the course of a measurement. Following the respiratory determinations the specimens were removed from the reaction vessels, placed in small plastic containers, and weighed to the nearest milligram. For salamanders over about 250 milligrams only one animal constituted a sample; however, for the smaller specimens more than one was placed in each reaction vessel. Generally an experimental sample consisted of one large, two medium, or three small animals per reaction vessel.

Respiratory measurements were made both when the manometers and reaction vessels were not shaken, and when they were shaken in the usual manner (135 oscillations per minute through a 5-cm. excursion). In the results this is termed with, and without, mechanical stimulation. When no shaking was done, particular care was taken to keep the rate of gas exchange low (by using small samples) in order to insure that the rate of oxygen diffusion from the gas phase to the liquid phase did not become a limiting factor. Indeed, that this was accomplished, and that diffusion did not become limiting may be inferred from both the shape and the slope of the curves shown in Figures 1, 2, and 3.

Following an equilibration period of 15 to 20 minutes, manometer readings

were taken at 15-minute intervals for a period of two hours. To minimize variations between individual readings, these raw data were plotted and the hourly rate determined from the slope of the resulting line. Oxygen consumption is expressed in cubic millimeters per gram (wet weight) per hour.

The following two items should be mentioned: (1) no efforts were made to acclimate the salamanders to the temperature of the measurements, other than the equilibration period described above, and (2) rhythms were not taken into account, except that approximately one-half the measurements were made in the morning and one-half in the afternoon. Ralph (1957) has observed a diurnal rhythm of locomotor activity in *Plethodon cinereus* that is modified by an influence having a lunar frequency.

RESULTS

Influence of temperature and mechanical stimulation on oxygen consumption

There is recorded in Table I a summary of the mean rate of oxygen consumption ($\text{mm.}^3/\text{gm.}/\text{hr.}$), for each temperature and body size, of the three species

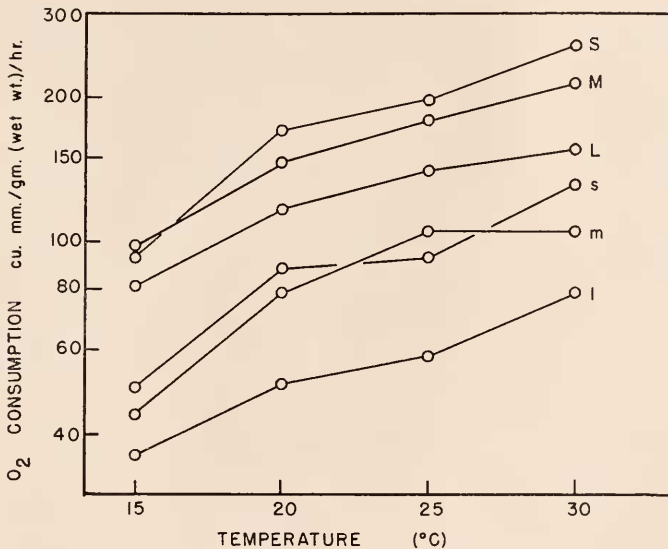


FIGURE 1. *Eurycea neotenes*: Relation of oxygen consumption to temperature and body size, plotted semi-logarithmically. S, M, L (small, medium, and large body size—mechanically stimulated). s, m, l (small, medium, and large body size—without mechanical stimulation).

of salamanders studied, both with and without mechanical stimulation. The number of measurements and standard deviations are shown. Figures 1, 2, and 3 present these data in graphic form. It is evident that *Eurycea neotenes* (Fig. 1) shows an increased rate of oxygen consumption at each higher temperature. The most rapid increase occurs between 15 and 20° C., while the rate of increase between 20 and 30° C. is somewhat less but appears to be more regular. Figure 2 for *Eurycea nana* reflects a fairly regular increase in the rate of oxygen consumption from 15° to a maximum at 25° C.; however, the curves reverse at 25° C. and

TABLE I
Mean oxygen consumption rate for three species of salamanders
 (mm.³/gm./hr.)

Animal size (Range in gm.)	Temperature (° C.)											
	15			20			25			30		
	Still	Shaken	Shaken	Still	Shaken	Shaken	Still	Shaken	Shaken	Still	Shaken	Shaken
<i>Eurycea nana</i>												
Small (0.01-0.1)	5/58 ± 14.5*	5/89 ± 10	5/77 ± 16.1	6/131 ± 51.2	10/106 ± 29.7	24/199 ± 37.9	—	—	—	—	—	8/183 ± 30.8
Med. (0.1-0.208)	6/48 ± 9.5	5/68 ± 15.8	3/71 ± 18.1	5/117 ± 26	8/86 ± 25	22/160 ± 31.8	—	—	—	—	—	8/158 ± 26.3
Large (0.216-0.431)	5/40 ± 4.9	5/49 ± 2.8	9/55 ± 16.9	9/92 ± 20.2	15/81 ± 18.1	19/155 ± 20.6	—	—	—	—	—	6/144 ± 25.9
<i>Eurycea neotenes</i>												
Small (0.101-0.148)	6/50 ± 9.5	4/93 ± 7.8	8/88 ± 26.3	7/170 ± 16	5/93 ± 8	4/197 ± 9	3/132 ± 7.1	5/253 ± 45.7				
Med. (0.159-0.327)	4/44 ± 8.1	4/98 ± 21.3	4/79 ± 18.1	6/146 ± 23.2	4/106 ± 7.9	3/179 ± 31.8	4/105 ± 6.2	5/214 ± 24.6				
Large (0.756-0.849)	6/36 ± 10	5/81 ± 9.7	6/51 ± 10.9	5/117 ± 21	3/58 ± 3.1	4/141 ± 4.2	4/79 ± 12.9	5/157 ± 12.9				
<i>Eurycea pterophila</i>												
Small (0.034-0.090)	3/78 ± 7.8	4/142 ± 12	4/134 ± 31.6	7/225 ± 28.1	4/97 ± 4.4	4/202 ± 15.4	4/91 ± 19.7	4/182 ± 13.7				
Med. (0.111-0.280)	4/84 ± 24.3	5/134 ± 21.5	6/116 ± 33.3	5/179 ± 25.9	5/90 ± 15.6	4/161 ± 19.3	4/82 ± 15.7	4/156 ± 23.3				
Large (0.280-0.592)	4/66 ± 21.1	5/109 ± 24.7	4/85 ± 16.6	4/147 ± 15.9	3/65 ± 8.1	4/145 ± 22.6	3/62 ± 4.9	4/137 ± 22.6				

* The number of measurements precedes the slant. Standard deviations are shown.

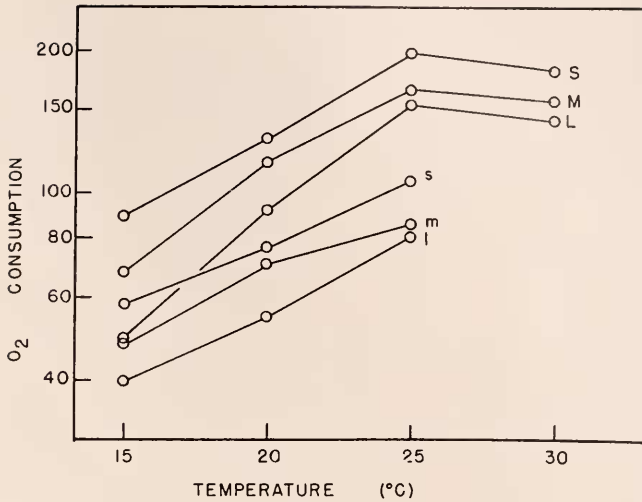


FIGURE 2. *Eurycea nana*: Relation of oxygen consumption to temperature and body size, plotted semi-logarithmically. S, M, L (small, medium, and large body size—mechanically stimulated). s, m, l (small, medium, and large body size—without mechanical stimulation).

a decreasing rate is shown between 25° and 30° C. The results obtained with *Eurycea pterophila* are revealed in Figure 3. Following a rapid increase in rate of oxygen consumption from 15° to a maximum at 20° C., the curves reverse their slope and show a decreasing rate from 20 to 30° C.

It is striking that mechanical stimulation does not alter the shape of the curves but merely shifts them up to the rate axis, *i.e.*, the same conclusions are apparent from either set of curves: those yielded when the organisms were mechanically stimulated by shaking, or those obtained when the reaction vessels were motionless.

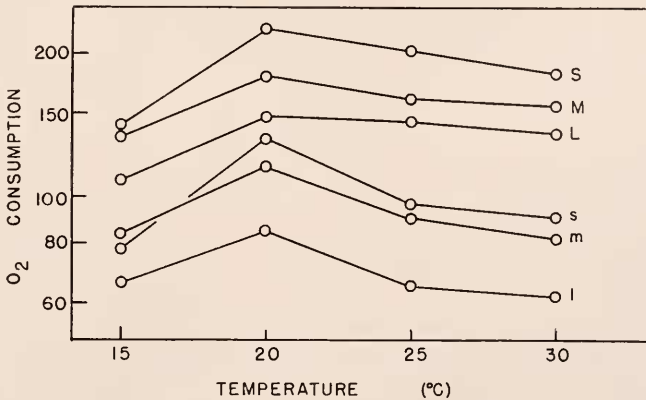


FIGURE 3. *Eurycea pterophila*: Relation of oxygen consumption to temperature and body size, plotted semi-logarithmically. S, M, L (small, medium, and large body size—mechanically stimulated). s, m, l (small, medium, and large body size—without mechanical stimulation).

Relation of body size to oxygen consumption

These data are amenable to some analysis of the effect of body size on the rate of oxygen consumption. In Table I and Figures 1, 2, and 3, the data were divided arbitrarily into three groups (small, medium, and large) on the basis of body size. It is obvious that the highest rate of oxygen consumption is shown by the smaller organisms, while the lowest rate is exhibited by the largest salamanders, and an intermediate rate is shown by medium-sized specimens. Again it is apparent that this may be observed from either set of curves, *i.e.*, the ones obtained with mechanical stimulation, or those resulting without shaking.

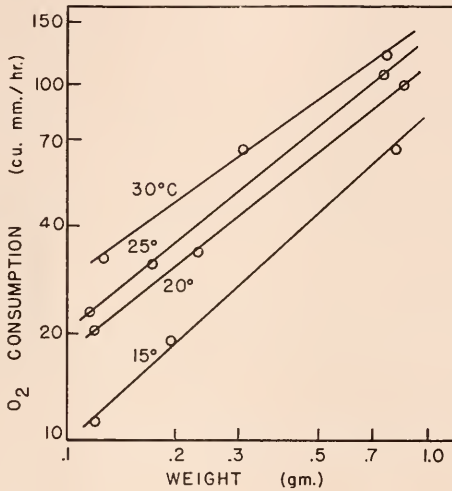


FIGURE 4. Plot of oxygen consumption *vs.* body weight for mechanically stimulated *Eurycea neotenes* at 15, 20, 25, and 30° C. (log-log plot).

Utilizing the equation given by Prosser and Brown (1961) for the relation of total metabolism to body size,

$$M = KW^b$$

$$\log M = b \log W + \log K$$

where M = total metabolism, or O_2 consumed per hour

W = body weight

b is given by the slope of the plot of $\log O_2$ consumption against \log weight

K is obtained from the Y intercept,

a further treatment of the data is possible. Figure 4 contains a plot, for mechanically stimulated *Eurycea neotenes*, of the logarithm of oxygen consumed per hour against the logarithm of the weight of the organism. Typical progression curves are yielded. The approximate values of "b" at various temperatures, as calculated from the slope of the lines, not only for *Eurycea neotenes*, but also for the other two species of salamanders studied, are presented in Table II.

TABLE II

Values of "b" for three species of salamanders at different temperatures

Temperature	<i>Eurycea nana</i>	<i>Eurycea neotenes</i>	<i>Eurycea pterophila</i>
15° C.	.68	.92	.83
20° C.	.80	.81	.75
25° C.	.79	.82	.80
30° C.	.85	.73	.80

Effect of oxygen tension on the rate of oxygen consumption

Prosser (1955) mentions that oxygen availability has not been thoroughly investigated as a factor limiting the range of species or isolating populations. With this in mind the following preliminary experiments were undertaken. Various mixtures of oxygen and nitrogen were prepared as described by Umbreit *et al.* (1957), and after the salamanders were placed in the reaction vessels and attached

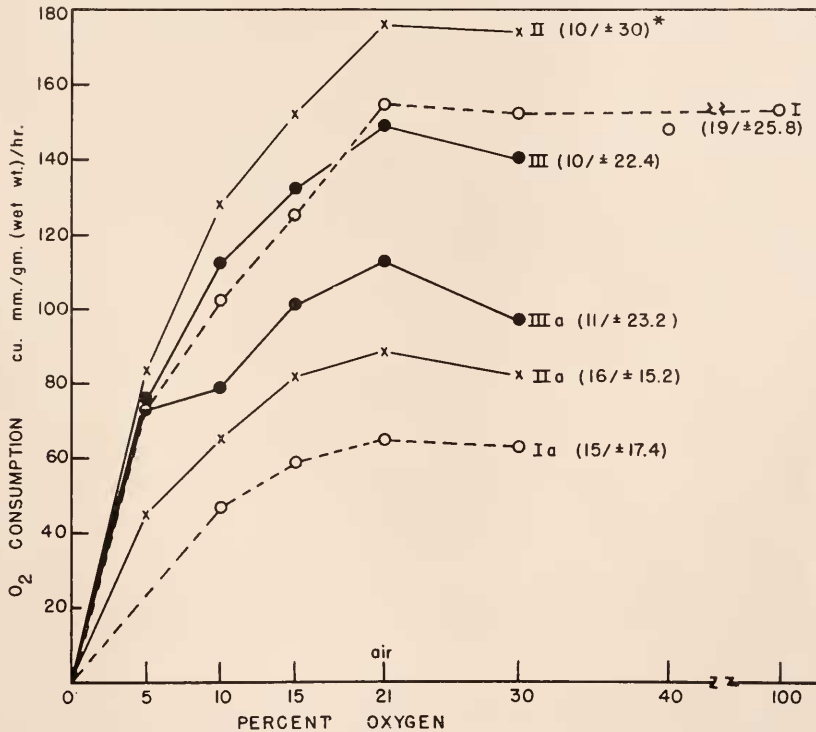


FIGURE 5. Influence of oxygen concentration on rate of oxygen consumption by mechanically stimulated salamanders at two different temperatures. I (dotted line, open circles), *Eurycea nana*; II (crosses), *Eurycea neotenes*; III (closed circles) *Eurycea pterophila*. I, II, III at 30° C.; Ia, IIa, IIIa at 15° C. *Numbers in parentheses indicate number of measurements and standard deviation of the point plotted for air. All other points are the average of 2 to 5 measurements.

to the manometers, the gas atmosphere was changed by flushing each vessel with at last one liter of the desired gas mixture.

Curves relating the rate of oxygen consumption to the per cent oxygen in the gas mixture are presented in Figure 5. Although the curves are not as smooth as might be desired (possibly due to a relatively small number of measurements averaged for each point, other than air), it is quite clear that the usual type of hyperbolic curves result. The maximum rate of oxygen consumption occurs in air in each case. Below the oxygen content of air the rate of oxygen consumption is related to oxygen availability. Just why a slightly decreased rate of oxygen consumption is shown in an atmosphere of 30% oxygen is puzzling; however, due to the limited number of determinations made under these conditions, as well as the standard deviation exhibited by the points plotted for air (see Fig. 5), this decrease is not considered significant.

DISCUSSION

The fact that the highest rate of oxygen consumption shown by the salamanders investigated occurs at a different temperature for each species is most interesting. Specifically, the maximal rate for *Eurycea neotenes* occurs at 30° C., for *Eurycea nana* at 25° C. and for *Eurycea pterophila* at 20° C. Mechanical stimulation (shaking) causes approximately a two-fold increase in the rate of oxygen consumption (Figs. 1, 2, 3). Mechanical stimulation does not change the shape of the R/T curves, but only relocates them higher on the rate axis.

The correlation of these results with natural habitat conditions of the salamanders may be of importance. There is briefly summarized in Table III information pertaining to the habitat. In nature *Eurycea nana* is subjected to the least variable temperature and the lowest oxygen content. *E. nana* shows maximal oxygen consumption at 25° C., which is slightly above the habitat temperature, but oxygen uptake is decreased at a temperature of 30° C. With regard to *E. nana* a qualitative observation was made which seems pertinent. In measuring oxygen consumption (no mechanical stimulation), it was noted that about one-half the organisms died when subjected to a temperature of 30° C. for a little over two hours. Of course in these cases no data were obtained. At 25° C. about one out of every seven animals died. Mechanical stimulation lessened the mortality, as only about one out of ten perished at 30° C. and none died at 25° C. For the other two species of salamanders no lethal effects due to the higher temperatures were observed. This seems particularly unique in the case of *E. pterophila* which exhibits a maximal respiratory rate at 20° C. These observations may mean that *E. nana* is the least tolerant of temperature change, which is in accord with the almost constant temperature of its natural habitat.

Just why *E. pterophila* should show a maximal metabolic rate at 20° C., with a decreased oxygen consumption both at 25° and 30° C., while *E. nana* shows a maximal rate at 25° C., and a decreased rate at 30° C., is not clear, particularly when the temperature of the natural habitat is essentially the same in both cases. Possibly the explanation lies in different characteristics of the enzymatic machinery of the two species. With regard to enzyme activity it is clear that as temperature is increased two forces come into play: the rate of

destruction of the enzyme, versus the increase in rate of substrate transformation; and at elevated temperatures, the first may overshadow the second.

The influence of temperature on the metabolism of *Eurycea neotenes* is straightforward in that oxygen consumption shows a fairly regular increase with each increase in temperature throughout the range investigated. This correlates nicely with the wider temperature variation that *E. neotenes* encounters in nature (Table III).

As pointed out by Prosser and Brown (1961), interpretation of the size correlations of metabolism are difficult. In general, small animals exhibit a higher standard metabolic rate than related animals of greater body weight (Zeuthen, 1953; Davison, 1955). The salamanders included in this study represent no

TABLE III
Habitats of the three species of salamanders studied

Species	<i>Eurycea nana</i>	<i>Eurycea pterophila</i>	<i>Eurycea neotenes</i>
Locality	Spring Lake, San Marcos, Tex.	Fern Bank Springs, near the Blanco River. Eight miles west of San Marcos, Texas	Prof. Green's Place. Near Devil's Backbone. Fifteen miles southwest of San Marcos, Texas.
Description	Spring-fed, clear deep water. Abundant aquatic vegetation.	Natural springs. Clear, shallow, moderately fast flowing water. Abundant aquatic vegetation.	A spring-fed, creek or stream type habitat. Slower moving water. Somewhat less aquatic vegetation.
Temperature (Throughout a six-month period, Feb. to August.)	21-22° C.	21-22.5° C.	19-23.5° C.
Oxygen content*	3.2 ml./l.	5.7 ml./l.	4.9 ml./l.

* The figures reported are average values determined by Gary D. Henry (unpublished data). Analyses for oxygen were carried out at various times of the day over a period of several months, employing the apparatus and method described by Scholander *et al.* (1955).

exception to this rule, as may be seen from the data presented in Figures 1 through 4, as well as in Table II. Plotting the metabolism of species against body size on double logarithmic paper, Zeuthen (1953) has observed a continuous three-phase curve. For unicellular organisms the slope "*b*" of the curve is 0.7; for small metazoa, *b* is about 0.95; and for larger poikilothermic animals and for homeothermic animals *b* is approximately 0.75. Prosser and Brown (1961) state that *b* values vary from 0.55 to 1.0. Among the causes of this variation they mention body surface in homeotherms and in unicellular organisms, growth patterns, types of external respiration, increase of enzymes related to body mass, and disproportionate increase of different tissues. From Table II, *b* values for the various salamanders range between 0.68 and 0.92, with most of the values

falling between 0.73 and 0.85. Thus, there is good agreement between the values obtained in this study and those previously reported in the literature.

A correlation between the oxygen consumption of aquatic animals and their ecology has been frequently demonstrated. Usually, active species and inhabitants of rapid streams exhibit a higher rate of oxygen consumption than do more sluggish species and inhabitants of slow streams or standing water (Prosser *et al.*, 1950, Table 42). Often the inhabitants of oxygen-deficient water are able to maintain a steady rate of oxygen consumption when oxygen tension of the medium is falling, until a low critical level of oxygen is reached (Mann, 1956). Evans (1939) demonstrated many years ago that in some species of salamanders oxygen consumption was dependent on oxygen tension. There do not appear to be significant differences in the maximal rate of oxygen consumption exhibited by the three species of salamanders studied here, although the maximal rate does occur at different temperatures for the different species. Apparently, differences in habitat conditions (Table III) are not large enough to be reflected in the organisms' response to different concentrations of oxygen (Fig. 5). It is evident from the data of Figure 5 that below the oxygen content of air the rate of oxygen consumption is dependent on the oxygen concentration; however, this relationship is not strictly linear and thus these organisms are not "oxygen conformers" in the true sense of Prosser and Brown's (1961) definition. Neither are they very good "regulators," but probably they belong in that intermediate group of organisms between conformers and regulators.

SUMMARY

1. Three species of neotenic salamanders of the genus *Eurycea* were collected from their specific, though not widely separated, habitats on the escarpment region of the Edwards plateau in south central Texas.

2. Utilizing standard Warburg manometry, oxygen consumption was determined for each of the species (with and without shaking) at 15, 20, 25, and 30° C. Maximal respiratory rates were exhibited by *Eurycea neotenes* at 30° C., by *Eurycea nana* at 25° C., and by *Eurycea pterophila* at 20° C. Mechanical stimulation (shaking) increased the rate of oxygen uptake but did not alter the shape of the R/T curves. For *E. nana* and *E. neotenes* the temperature at which the maximal metabolic rates were observed appeared to be correlated with the temperature characteristics of the natural environment.

3. There is a decrease in metabolic rate with increasing body size. The specific relationship is in good agreement with previously reported values.

4. All three species of salamanders seem to be intermediate between oxygen conformers and regulators, in that the rate of oxygen consumption is dependent on oxygen concentration (up to the oxygen content of air), but not in a strictly linear manner.

LITERATURE CITED

- BAKER, J. K., 1961. Distribution of and key to the neotenic *Eurycea* of Texas. *Southwestern Nat.*, 6: 27-32.
- BOELL, E. J., 1955. Energy exchange and enzyme development during embryogenesis. In: *Analysis of Development*. B. H. Willier, P. A. Weiss and V. Hamburger, eds. Pp. 520-555. W. B. Saunders Co., Philadelphia.

- BULLOCK, T. H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.*, **30**: 311-342.
- DAVISON, J., 1955. Body weight, cell surface, and metabolic rate in anuran Amphibia. *Biol. Bull.*, **109**: 407-419.
- EVANS, G., 1939. Factors influencing the oxygen consumption of several species of Plethodontid salamanders in aerial and aquatic media. *Ecology*, **20**: 74-95.
- FOX, H. M., AND C. A. WINGFIELD, 1937. The activity and metabolism of poikilothermal animals in different latitudes. II. *Proc. Zool. Soc. London, Ser. A.*, **107**: 275-282.
- LØVTRUP, S., AND B. WERDINIUS, 1957. Metabolic phases during amphibian embryogenesis. *J. Exp. Zool.*, **135**: 203-220.
- MANN, K. H., 1956. A Study of the oxygen consumption of five species of leech. *J. Exp. Biol.*, **33**: 615-626.
- PROSSER, C. L., 1955. Physiological variation in animals. *Biol. Rev.*, **30**: 229-262.
- PROSSER, C. L., AND F. A. BROWN, JR., 1961. Comparative Animal Physiology. 2nd Edition. W. B. Saunders Co., Philadelphia.
- PROSSER, C. L., D. W. BISHOP, F. A. BROWN, JR., T. L. JAHN AND V. J. WULFF, 1950. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia.
- RALPH, C. L., 1957. A diurnal rhythm in *Plethodon cinereus* and its modification by an influence having a lunar frequency. *Biol. Bull.*, **113**: 188-197.
- SCHOLANDER, P. F., L. VAN DAM, C. L. CLAFF AND J. W. KANWISHER, 1955. Micro gasometric determination of dissolved oxygen and nitrogen. *Biol. Bull.*, **109**: 328-334.
- UMBREIT, W. W., R. H. BURRIS AND J. F. STAUFFER, 1957. Manometric Techniques. Burgess Publishing Co., Minneapolis, Minnesota.
- VERNBERG, F. J., 1952. The oxygen consumption of two species of salamanders at different seasons of the year. *Physiol. Zoöl.*, **25**: 243-249.
- VERNBERG, F. J., 1959a. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull.*, **117**: 163-184.
- VERNBERG, F. J., 1959b. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. III. The influence of temperature acclimation on oxygen consumption of whole organisms. *Biol. Bull.*, **117**: 582-593.
- WIENS, A. W., AND K. B. ARMITAGE, 1961. The oxygen consumption of the crayfish *Orconectes immunis* and *Orconectes nais* in response to temperature and to oxygen saturation. *Physiol. Zoöl.*, **34**: 39-54.
- ZEUTHEN, E., 1953. Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, **28**: 1-12.